Effects of Soy Consumption on Gonadotropin Secretion and Acute Pituitary Responses to Gonadotropin-Releasing Hormone in Women[1,2]

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ABSTRACT Soy contains the isoflavone phytoestrogens, genistein and daidzein. These isoflavones are partial estrogen agonists in cell and animal models, but effects from dietary soy in humans are unclear. Experiments were conducted in pre- and postmenopausal women to examine whether dietary isoflavones from soy behave as estrogen agonists, antagonists or have no effect on the estrogen-sensitive pituitary. Pituitary sensitivity to gonadotropin-releasing hormone (GnRH), an estrogen-sensitive endpoint, was measured during GnRH challenge tests administered before, during and after dietary soy consumption. The response to an isoflavone-rich soy food diet was examined in five premenopausal and seven postmenopausal women using transdermal estrogen replacement therapy. Estrogen agonists suppress gonadotropin concentrations and enhance GnRH priming (enhanced gonadotropin secretion in response to repeated doses of GnRH), whereas antagonists elevate gonadotropin concentrations and have no effect on GnRH priming. Each subject consumed 50 g textured soy protein containing 60 mg total isoflavones daily for 10–14 d. Baseline estradiol concentrations were consistent among study periods. In both pre- and postmenopausal women, soy consumption did not affect mean baseline or peak luteinizing hormone (LH) concentrations, indicating a lack of estrogen-like effect at the level of the pituitary. However, in postmenopausal subjects, mean LH secretion decreased after discontinuing soy, suggesting a residual estrogenic effect. In one premenopausal woman, enhanced LH secretion was observed after soy treatment, suggesting there may be subpopulations of women who are highly sensitive to isoflavones. J. Nutr. 132: 708–714, 2002.

KEY WORDS: • *soy* • *phytoestrogen* • *isoflavone* • *pituitary* • *humans*

Early interest in the possible estrogen-like effects of soy was prompted by the discovery of extremely high levels of isoflavones, a class of dietary derived nonsteroidal estrogens, in urine and blood after soy food consumption (1), and epidemiologic data showing that women in Asia have a low incidence of breast cancer, a disease that is estrogen-dependent and influenced by diet (2–4). Soy is regularly consumed in many Asian countries, and soybeans are the richest known source of the isoflavone phytoestrogens, genistein and daidzein (5–7). Genistein and daidzein are partial estrogen agonists and antagonists; on the basis of its conformational binding to the estrogen receptor (ER)[5] (8), genistein can be classified as a selective estrogen receptor modulator.

After soy consumption, circulating isoflavone concentrations increase significantly in humans (1,9). Isoflavones can have clear biological effects on estrogen target cells in vitro (10–12) and affect the reproductive system of several animal species in vivo (13–17). Demonstrating estrogen agonist or antagonist effects from soy isoflavones in humans is more difficult, but studies have indicated that diets rich in soy isoflavones can alter menstrual cycle length and influence endogenous estrogen metabolism (18,19). Whether soy has sufficient estrogen-like effects to treat postmenopausal estrogen deficiency remains controversial (20).

Observing estrogen-like changes in hormone patterns in humans is complicated by a tightly linked hypothalamic-pituitary-ovarian feedback system that maintains endogenous estrogen within physiologic limits. In addition, among mammals, humans have relatively high concentrations of

[1] Presented in poster form at the First International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, February 20–23, 1994, Mesa, Arizona; the poster abstract was published in the meeting proceedings [Nicholls, J., Lasley, B. L., Gold, E. B., Nakajima, S. T., & Schneeman, B. O. (1995) Phytoestrogens in soy and changes in pituitary response to GnRH challenge tests in women. J. Nutr. 125: 803S (abs.)].

[2] Funded in part by grants from the U.C. Davis College of Agriculture and Environmental Sciences, the U.C. Davis Clinical Nutrition Research Unit and the U.C. Davis Collaborative Research Grants Program. J.N. was supported in part by a grant from the U.S. Department of Agriculture.

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[5] Abbreviations used: AUC, area under the curve; E2, estradiol; ER, estrogen receptor; ERT, estrogen replacement therapy; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; IU, international units standardized against WHO LH and FSH standards; LH, luteinizing hormone; post-T, post-treatment; pre-T, pre-treatment; T, treatment; TSP, textured soy protein.

Manuscript received 28 August 2001. Initial review completed 4 October 2001. Revision accepted 22 January 2002.

circulating estrogens and a high affinity estrogen-binding globulin. Consequently, modest levels of exogenous bioactive estrogens may not always result in predictable systemic changes in circulating hormone concentrations in women, but might have estrogen-like effects on specific target organs.

Because women consuming soy will be exposed concurrently to endogenous estrogens and isoflavones, the potential for interactions exists. The present study was designed to investigate the effect of dietary soy isoflavones on a specific and sensitive estrogen target cell, the pituitary gonadotroph. The sensitivity of the pituitary was examined using the gonadotropin-releasing hormone (GnRH) challenge test in healthy young premenopausal women and postmenopausal women using transdermal estrogen replacement therapy (ERT). Our goal was to determine whether dietary isoflavones, when administered against a constant background of estrogen, would act as estrogen agonists or antagonists, or whether they would be neutral with regard to effects on pituitary sensitivity.

SUBJECTS AND METHODS

Subjects. Potential research subjects were screened by questionnaire, including the Health Habits and History Questionnaire food-frequency form (21) and a personal history form. Subjects were excluded if they were vegans, habitual consumers of soy foods, smokers or heavy exercisers, or they had known systemic disorders. Subjects enrolled were omnivores and had normal height for weight based on calculation of body mass index. All had normal liver, kidney and hematologic functions as confirmed by clinical laboratory tests. None used antibiotics in the 4 wk before or during the study period.

Premenopausal subjects ($n = 5$) taking oral contraceptives or other drugs known to alter hormone status were excluded. Subject #104 stopped taking oral contraceptives 3 mo before starting the study.

Postmenopausal subjects ($n = 7$) were hypogonadal, either due to surgical or natural menopause, and all had undergone hysterectomies. All subjects had been using transdermal ERT (Estraderm patch, 0.05 mg/d, twice weekly, donated by Wyeth-Ayerst for the duration of the study) for at least 3 mo before the study, and continued on this regimen during the study. This population was chosen to minimize variations in serum estradiol (E2) concentrations that occur in premenopausal women, or during combined hormone replacement therapy (estrogen plus progestin), which is standard therapy for postmenopausal women without hysterectomies. All subjects who had undergone surgical menopause had nonhormonally mediated indications for their hysterectomy and/or oophorectomy. Other than Estraderm, subjects were taking no other drugs known to alter hormone status. The demographics of the two study groups are shown in **Table 1**. The protocol was approved by the University of California, Davis Institutional Review Board, and informed consent was obtained.

Study design. For all subjects, the study design consisted of sequential pretreatment (pre-T), dietary treatment (T) and post-treatment (post-T) periods, each of which was followed immediately by a GnRH challenge test. Studies have shown that latent effects of soy consumption may continue some time after discontinuing soy (18,19). To test this possibility, the post-T period, during which no soy foods were consumed, was included.

During all study periods, body weight and daily dietary intakes were recorded. Subjects were given a diet scale and instructed how to keep accurate records. Diet records were analyzed for nutrient content using Nutritionist III dietary analysis software. After pre-T, habitual intakes of dietary energy, carbohydrate, protein, fat and alcohol were determined. During T, subjects consumed a total of 50 g/d textured soy protein (TSP) in one muffin and one patty, each containing 25 g TSP. Based on diet records collected during pre-T, subjects were counseled as to which regularly consumed foods should be replaced by the TSP-containing foods to maintain energy and macronutrient intake consistent among study periods.

The 50 g TSP used in the present study was analyzed by HPLC and found to contain 60 mg of total isoflavones, mainly in the form of genistein, daidzein, daidzin and genistin (22). Each subject consumed soy from the same batch of TSP throughout the study (Guisto's, South San Francisco, CA).

TABLE 1

Subject characteristics at onset of study

Subject #	Age (*y*)	Height (*cm*)	Weight (*kg*)	Body mass index (kg/m^2)
Premenopausal				
101	32	163	52.3	19.8
102	35	178	61.4	19.4
103	34	157	54.1	21.8
104	28	163	47.7	18.1
105	25	160	50.9	19.9
Mean ± SEM	30.8 ± 1.9	164.4 ± 4	53.3 ± 2.3	19.8 ± 0.6
Postmenopausal				
1	59	163	73.2	27.7
2	36	163	57.3	21.7
3	51	165	62.3	22.8
4	47	165	54.5	20.0
5	52	160	57.8	22.2
6	55	170	68.2	23.5
7	46	165	65.0	23.8
Mean ± SEM	49 ± 3	164 ± 1	62.2 ± 2.5	23.1 ± 0.9

On the last day of each study period, a complete 24-h urine collection was obtained for the measurement of urinary isoflavone output. Samples were refrigerated at all times during the collection day; on the day after collection, 50-mL aliquots were stored at −70°C until analysis. Urinary isoflavones were measured by gas chromatography/mass spectrometry (1). On the day immediately after each study period, each subject underwent a GnRH challenge test.

For premenopausal subjects, the duration of the study was ~3 mo (**Fig. 1**). Pre-T lasted 7 d, T lasted 14 d and post-T lasted 7 d. All study periods were scheduled to end on d 5 or 6 of the subject's menstrual cycle to coincide with the early follicular phase, during which studies have shown that GnRH priming can be observed (23,24,25). The timing also ensured that the GnRH challenge tests were administered against similar E2 concentrations.

To predict the approximate day of the onset of menstruation (cycle d 1), urinary luteinizing hormone (LH) was monitored starting on d 9–11 of the menstrual cycle preceding each study period (Ovu-QUICK ovulation predictor kit, Quidel, San Diego, CA). When the LH surge was detected, d 1 of menstruation was estimated to begin 14 d later (26). Soy treatment began 5–6 d after detection of the LH surge and lasted until the day before the GnRH challenge. The range for lengths of treatment periods was 13 to 19 d (14.6 ± 2.5, mean ± SD, $n = 5$). Diet records were kept daily during each study period and 3 d/wk between study periods. Analysis of dietary records was performed on the 7 d of pre-T and post-T, and during the final 7 d of T.

For postmenopausal subjects, the study lasted 30 d. Pre-T lasted 7 d, T lasted 10 d, post-T lasted 7 d and each period was followed by a GnRH challenge. Daily diet records were kept throughout, except for GnRH challenge days. The 7 d of pre-T and post-T and the final 7 d of T were analyzed for nutrient intake.

GnRH challenge. The GnRH challenge test is a sensitive and dynamic test that measures changes in responsiveness of the estrogen-sensitive hypothalamic-pituitary axis to estrogen agonists and antagonists (23–25). The GnRH challenge has proved effective in measuring changes in pituitary sensitivity to GnRH as E2 concentrations change during the menstrual cycle, and we developed a protocol to allow the measurement of changes due to the presence of dietary estrogens.

On the days of the GnRH challenge tests, subjects consumed a small meal 3 h before the start of the tests. Baseline venous blood samples (5 mL) were obtained at −30 min, −15 min and at time zero before the initial 10-μg intravenous bolus of GnRH. Thereafter,

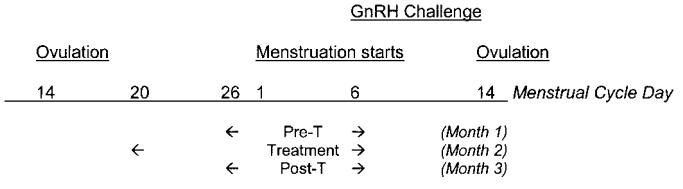

FIGURE 1 Study design for premenopausal women (figure shows end of one and beginning of second 28-d cycle). The pretreatment period lasted 7 d, soy treatment lasted 14 d and post-treatment lasted 7 d, during which diet records were kept daily. Study periods were scheduled so that gonadotropin-releasing hormone (GnRH) challenges fell on d 5 or 6 of three consecutive menstrual cycles (start of menstruation = cycle d 1). Ovulation was monitored midcycle (14 d later) to estimate cycle d 1, and study periods were scheduled accordingly. Between study periods, diet records were kept every 3 d.

blood samples were collected every 15 min for 120 min before the second 10-μg intravenous bolus of GnRH was administered. Samples were collected every 15 min for an additional 120 min. To ensure a consistent circulating E2 concentration for each GnRH challenge test, postmenopausal subjects applied a fresh Estraderm patch 2 d before each test. Baseline hormone concentrations were measured in samples drawn before the initial GnRH dose. In samples drawn after GnRH was given, LH and follicle-stimulating hormone (FSH) were measured in every sample, and E2 and progesterone were measured in every fourth sample. Hormone samples from each subject were analyzed together using standard double antibody RIA kits (Diagnostic Products, Los Angeles, CA). Detection limits were 8000 ng/L, 30 mg/L, 2 IU/L and 1.6 IU/L for E2, progesterone, LH and FSH, respectively. Intra-assay variability was 7.0 and 6.4%, and interassay variability was 8.1 and 10.0% for E2 and progesterone, respectively. For premenopausal subjects, intra-assay variability was 7.0 and 3.1%, and interassay variability was 7.9 and 7.7% for LH and FSH, respectively. For postmenopausal subjects, intra-assay variability was 1.8 and 3.9% and interassay variability was 4.0 and 4.2% for LH and FSH, respectively.

Statistical analysis. Sample sizes were chosen on the basis of experiments in which significant differences in gonadotropin peak height during GnRH challenge tests were seen in groups of six women (24). Statistical analyses were performed using StatView 512 software. Results are expressed as means ± SEM and differences assessed using repeated-measures ANOVA and Fisher's LSD multiple comparison test to compare values obtained during the three study periods. A log transformation was made when there was unequal variance. Differences were considered significant when $P < 0.05$.

RESULTS

Dietary intake and isoflavone excretion. No differences among study periods were observed for daily energy intake or percentage of energy intake from carbohydrate, protein, fat or alcohol in either subject population (**Table 2**).

Urinary genistein and daidzein excretions were higher after soy consumption compared with after pre-T in both study populations, confirming adherence to the soy-containing diet (**Table 3**). One pre- and two postmenopausal subjects excreted detectable, but low concentrations of equol, a biologically active isoflavone metabolite. Isoflavone excretion by individual subjects was highly variable, as has been reported in other studies (1,27–31). In postmenopausal subjects, genistein and daidzein excretion varied approximately sixfold, whereas in premenopausal subjects, genistein and daidzein excretion varied three- to fourfold.

Hormones. The gonadotropin releasing hormone (GnRH) challenge test was employed to measure changes in pituitary sensitivity. Gonadotropin secretion in response to GnRH was highly variable among subjects in both study groups. For most subjects, obvious LH peaks were evident, whereas FSH lacked a clear pattern of secretion in response to GnRH. Therefore, analyses included baseline concentrations of E2, progesterone, LH and FSH (Table 3), and LH secretion in response to GnRH (**Fig. 2**, **Fig. 3** and **Table 4**). Mean peak LH data are expressed as increment from baseline and as percentage of baseline to show dependence of peak height on baseline LH concentration. The area under the curves (AUC) of the two LH peaks were measured individually starting at respective LH concentrations at time zero (Peak I) and 120 min (Peak II), and were identified as Area I and Area II. Total Area consists of AUC under both peaks measured from baseline concentration at time zero.

For premenopausal subjects, baseline hormone concentrations for each study period were calculated as the mean of values measured in three samples collected at −30 min, −15 min and time zero. For postmenopausal subjects, baseline concentrations were calculated as the mean of values measured in two samples collected at −30 min and time zero. Serum E2, progesterone, LH and FSH baseline hormone concentrations measured after the three study periods did not differ in either subject population (Table 3).

In premenopausal subjects, a consistent mean E2 baseline concentration of ~30 ng/L was maintained for the three study periods. In postmenopausal subjects, consistent mean E2 baseline concentrations between 35 and 45 ng/L, within the expected range for the early follicular phase of the menstrual cycle, were maintained for the three study periods.

In individual premenopausal subjects, LH Peaks I and II occurred 15 or 30 min after GnRH administration. No differences in the gonadotropins were observed among the three study periods in premenopausal subjects for any peaks, whether expressed as increment from baseline, percentage of baseline (Fig. 2) or AUC (Table 4).

In individual postmenopausal subjects, LH Peak I occurred

TABLE 2

Nutrient intake of pre- and postmenopausal women during pretreatment, soy treatment and post-treatment periods[1,2]

Nutrient	Pretreatment	Treatment	Post-treatment
		Premenopausal	
Energy, *kJ*	7,188 ± 490	8,263 ± 356	7,799 ± 845
Energy, *kcal*	1,718 ± 117	1,975 ± 85	1,864 ± 202
Carbohydrate, *%en*	52 ± 3	53 ± 2	53 ± 2
Protein, *%en*	15 ± 1	16 ± 1	16 ± 1
Fat, *%en*	30 ± 4	29 ± 2	28 ± 2
Alcohol, *%en*	3 ± 1	2 ± 1	3 ± 1
		Postmenopausal	
Energy, *kJ*	6,502 ± 452	6,962 ± 335	6,636 ± 561
Energy, *kcal*	1,554 ± 108	1,664 ± 80	1,586 ± 134
Carbohydrate, *%en*	56 ± 2	56 ± 2	54 ± 2
Protein, *%en*	17 ± 1	17 ± 1	16 ± 1
Fat, *%en*	26 ± 2	26 ± 2	29 ± 2
Alcohol, *%en*	2 ± 1	1 ± 0	1 ± 0

[1] Values are means ± SEM; premenopausal $n = 5$; postmenopausal $n = 7$.

[2] No differences were observed for any nutrient among study periods, $P \geq 0.05$; %en, % energy.

TABLE 3

Urinary isoflavone and serum steroid and baseline gonadotropin concentrations in women after pretreatment, soy treatment and post-treatment periods[1–5]

	Pretreatment	Treatment	Post-treatment
		Premenopausal	
Urinary isoflavones, *mg/d*			
Genistein	1.53 ± .09[a]	3.59 ± 0.07[b]	1.94 ± 0.22[a]
Daidzein	1.03 ± 0.12[a]	4.18 ± 0.12[c]	2.62 ± 0.21[b]
Serum hormones			
Estradiol, *ng/L*	32.9 ± 7.0	29.4 ± 5.4	30.1 ± 5.3
Progesterone, *mg/L*	0.44 ± 0.11	0.41 ± 0.09	0.41 ± 0.10
LH, *IU/L*	6.4 ± 1.7	6.4 ± 1.7	5.9 ± 1.2
FSH, *IU/L*	13.2 ± 1.7	12.9 ± 1.1	12.2 ± 1.2
		Postmenopausal	
Urinary isoflavones, *mg/d*			
Genistein	2.03 ± 0.16[a]	3.45 ± 0.12[b]	2.13 ± 0.14[a]
Daidzein	1.89 ± 0.39[a]	4.16 ± 0.12[b]	2.31 ± 0.24[a]
Serum hormones			
Estradiol, *ng/L*	35.2 ± 2.3	44.2 ± 9.0	42.2 ± 7.3
Progesterone, *mg/L*	0.22 ± 0.05	0.14 ± 0.03	0.17 ± 0.02
LH, *IU/L*	65.2 ± 3.1	60.7 ± 3.9	62.2 ± 4.6
FSH, *IU/L*	99.2 ± 5.9	95.9 ± 7.2	98.1 ± 8.9

[1] Values are means ± SEM; premenopausal $n = 5$; postmenopausal $n = 7$. LH, luteinizing hormone; FSH, follicle-stimulating hormone.

[2] Premenopausal hormone baselines were calculated as the mean of 3 values measured in samples collected at −30 min, −15 min and time zero.

[3] Postmenopausal hormone baselines were calculated as the mean of two values measured in samples collected at −30 min and time zero.

[4] For isoflavones, in premenopausal subjects, values are means ± SEM, $n = 4$ (subject #102 lacked a sample for pretreatment period; means include no data from subject #102). In postmenopausal subjects, values are means ± SEM, $n = 6$ (subject #2 lacked sample for pretreatment period; means include no data from subject #2). Because the variance was unequal, data were log-transformed before ANOVA.

[5] No differences were observed for any hormone among study periods, $P > 0.05$. Isoflavone values in a row with the same superscript are not different, $P \geq 0.05$.

30 or 45 min after GnRH administration, whereas Peak II was observed 15, 30 or 45 min after GnRH administration. The Peak I increment from baseline after pre-T was not significantly different from the increment after T; however, it was significantly higher than after post-T (Fig. 3). Peak II increments from baseline were significantly different among all study periods. However, when Peak II increments were measured from the LH concentration at 120 min, differences were no longer apparent (data not shown). When the same LH peaks were expressed as percentage of baseline, no differences were observed among the three study periods for Peaks I or II.

In postmenopausal subjects, as found for Peak I and II increments, no differences in AUC were observed between pre-T and T. However, LH tended to differ among study periods [for Area I ($P = 0.09$) and Total area ($P = 0.06$)]. This trend was based on a difference ($P < 0.05$) between pre-T and post-T. No differences were observed in Area II across study periods. Therefore, the AUC data agree with the peak data in

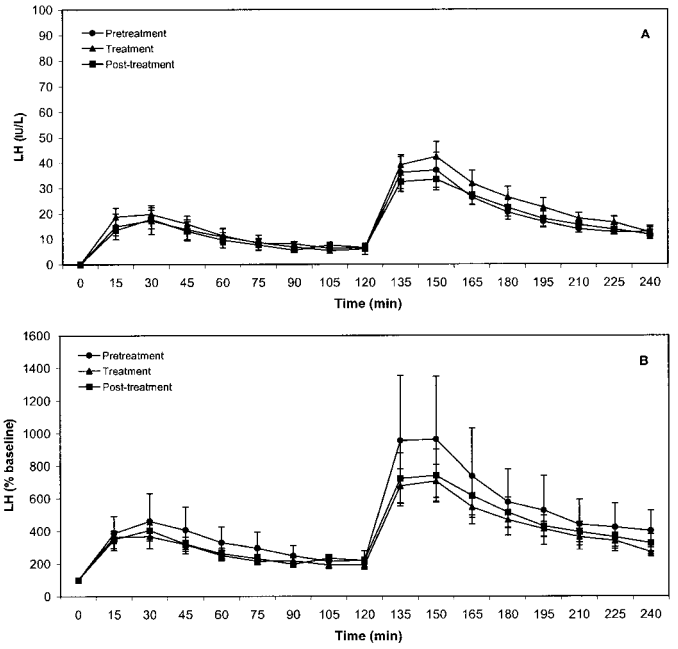

FIGURE 2 Serum luteinizing hormone (LH) in premenopausal women during gonadotropin-releasing hormone (GnRH) challenges after pretreatment, soy treatment and post-treatment periods. Values are means ± SEM, $n = 5$. LH is expressed as increment from baseline (*A*) or percentage of baseline (*B*). GnRH (10 μg) was administered intravenously at time zero and 120 min. LH baselines were calculated as the mean of values measured in samples collected at −30 min, −15 min and time zero.

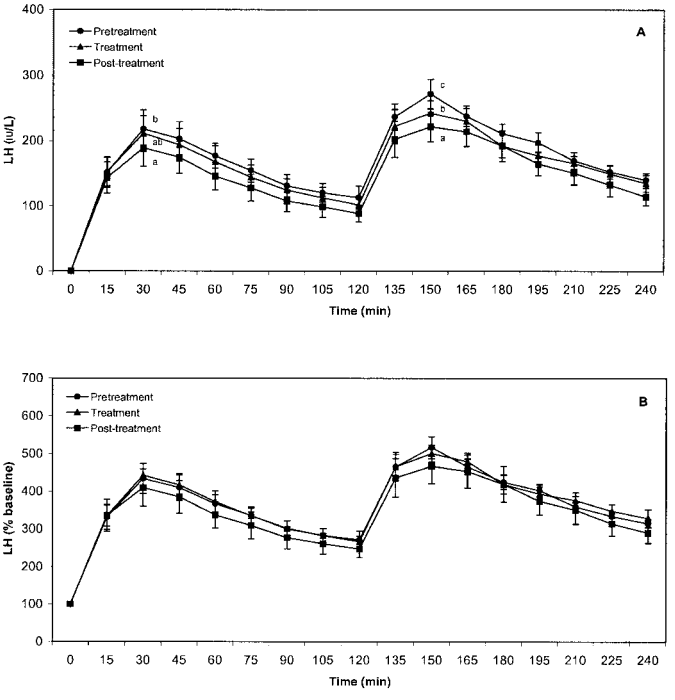

FIGURE 3 Serum luteinizing hormone (LH) in postmenopausal women during gonadotropin-releasing hormone (GnRH) challenges after pretreatment, soy treatment and post-treatment periods. Values are means ± SEM, $n = 7$. LH is expressed as increment from baseline (*A*) or percentage of baseline (*B*). GnRH (10 μg) was administered intravenously at time zero and 120 min. LH baselines were calculated as the mean of values measured in samples collected at −30 min and time zero. Means at a time without a common letter differ, $P < 0.05$.

TABLE 4

Serum luteinizing hormone peaks and AUC in women after pretreatment, soy treatment and post-treatment periods during GnRH challenge test[1–4]

	Pretreatment	Treatment	Post-treatment
		Premenopausal[5]	
LH area under the curve			
Area I, *IU/L × min*	1,206 ± 371	1,370 ± 230	1,172 ± 248
Area II, *IU/L × min*	1,967 ± 210	2,472 ± 361	1,817 ± 230
Total area, *IU/L × min*	3,794 ± 723	4,457 ± 646	4,324 ± 855
		Postmenopausal[6]	
LH area under the curve			
Area I, *IU/L × min*	18,134 ± 2,203[b]	17,404 ± 2,316[ab]	15,455 ± 2,345[a]
Area II, *IU/L × min*	10,515 ± 1,199	10,253 ± 882	9,894 ± 1,024
Total area, *IU/L × min*	42,195 ± 3,951[b]	39,850 ± 4,179[ab]	35,946 ± 4,672[a]

[1] Values are means ± SEM; premenopausal $n = 5$; postmenopausal $n = 7$. LH, luteinizing hormone.
[2] Gonadotropin releasing hormone (GnRH 10 μg) was administered intravenously at time zero and 120 min.
[3] Area I includes area under the curve (AUC) starting at LH concentration at time zero from zero to 120 min; Area II includes AUC starting at LH concentration at 120 min from 120 to 240 min; Total area includes AUC starting at LH concentration at time zero from time zero to 240 min.
[4] In premenopausal women, no differences were observed for any variable among study periods, $P > 0.05$.
[5] In postmenopausal women, values in a row with the same superscript are not different, $P < 0.05$. For Area I ($P = 0.09$) and Total Area ($P = 0.06$) LH tended to differ among study periods. This trend was based on a difference ($P < 0.05$) between pre-T and post-T.

postmenopausal subjects, showing that LH secretion in response to GnRH was significantly less after post-T compared with after pre-T.

DISCUSSION

The GnRH challenge test permits the evaluation of agonist and antagonist effects of an exogenous estrogenic stimulus on the estrogen-sensitive pituitary. It is a test of the sensitivity of the pituitary to release gonadotrophins when challenged with GnRH; when sufficient E2 is present to sensitize the pituitary, the test can detect interactions between circulating estrogenic substances. For both study populations, the endogenous steroidal estrogen concentrations were maintained relatively constant throughout the study periods (Table 3), improving the reliability of the test.

When E2 concentrations increase during the follicular phase of the menstrual cycle (d 1–14), the interplay between E2 and LH is complex. At low concentrations (d 1–4), E2 entrains LH secretion from the pituitary through negative feedback. As E2 concentrations increase toward midcycle (d 5–14), increasing E2 alters pituitary sensitivity to induce a positive feedback loop, causing increased LH secretion in response to GnRH. This positive feedback ultimately leads to the LH surge that precedes ovulation. The change in pituitary sensitivity that occurs in an estrogenic environment is measurable as GnRH priming, evidenced by enhanced LH secretion in response to repeated GnRH doses.

We hypothesized that if isoflavones were estrogen agonists, a diet containing soy isoflavones during the GnRH challenge would cause a suppressed baseline gonadotropin concentration and enhanced GnRH priming compared with control. Conversely, if isoflavones were estrogen antagonists, soy isoflavones would result in elevated gonadotropin baseline concentrations and GnRH priming would not occur.

In the premenopausal women, no changes in mean serum gonadotropin baseline concentrations, LH peaks or AUC were observed. These results indicate that, on average, exposure to dietary soy isoflavones had no effect on pituitary sensitivity. However, one subject exhibited a pattern of enhanced gonadotropin secretion during the GnRH challenge after consuming soy compared with after pre- and post-T. After soy, her baseline serum LH was elevated (data not shown), and her LH Peak I and II increments in response to GnRH were approximately double those seen after pre- and post-T (**Fig. 4**). The differences were less apparent when data were expressed as percentage of baseline, showing that the peaks were proportional to the increased baseline values. The response of this subject suggests that there may be a subpopulation of premenopausal women whose pituitaries are highly sensitive to the isoflavones in soy. Our study was not designed to determine factors that might contribute to this individual's different sensitivity to isoflavones, but it is interesting to note that she was the most physically active and among the leanest of the premenopausal subjects studied.

In the postmenopausal women, no changes in mean gonadotropin baseline concentrations were observed. An unexpected difference in mean serum LH peaks was observed after post-T during which soy had been discontinued. Peak LH secretion was suppressed compared with that observed in pre-T. This significant decrease in LH Peaks and Area I suggests that there may have been a residual estrogen agonist effect of soy consumption on LH concentrations.

Residual effects of dietary phytoestrogens have been reported previously. In premenopausal women, some studies have observed lengthening of the menstrual cycle that persisted for 2–5 mo after soy consumption was discontinued (18,19), although others have found no such effect (32). In postmenopausal subjects consuming soy flour, red clover sprouts and linseed sequentially for 2 wk each, an estrogenic effect on vaginal cytology was measurable 2 wk after dietary treatment stopped (33). However, no clinically relevant estrogen-like effects of soy on vaginal cytology or hormones were found in other larger studies of postmenopausal women consuming only soy (34,35).

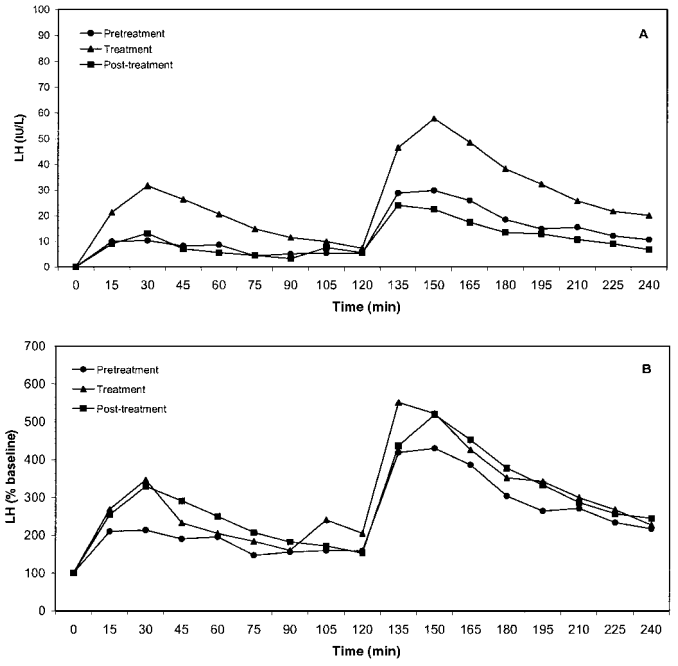

FIGURE 4 Serum luteinizing hormone (LH) in premenopausal subject #101 during gonadotropin-releasing hormone (GnRH) challenges after pretreatment, soy treatment and post-treatment periods. LH is expressed as increment from baseline (*A*) or percentage of baseline (*B*). GnRH (10 μg) was administered intravenously at time zero and 120 min. LH baselines were calculated as the mean of values measured in samples collected at −30 min, −15 min and time zero.

In postmenopausal subjects, no differences in GnRH priming were observed. Transdermal ERT brought E2 concentrations into the range seen in premenopausal women during the early follicular phase, and gonadotropins into normal ranges for transdermal ERT (36–38). However, the high serum LH in postmenopausal subjects indicates that LH secretion was not adequately entrained by the transdermal E2. Because evidence of GnRH priming requires that gonadotropins be partially entrained by E2, we were unable to observe GnRH priming. Given this hormonal environment, it is worth noting that the dietary isoflavones were not sufficiently estrogen-like to entrain the gonadotropins further. Although the sample size was small, results in the postmenopausal women suggest that at levels likely to be consumed in the diet, the intake of phytoestrogens may not be high enough to have an immediate estrogen-like effect on the pituitary.

In conclusion, consumption of 50 g TSP containing ~60 mg of the isoflavones genistein and daidzein for 10–14 d did not have a clear estrogen agonist or antagonist effect on pituitary sensitivity to GnRH in the pre- and postmenopausal women studied. The results indicate that dietary soy was relatively neutral on pituitary sensitivity, although there was a hint of an estrogen agonist effect. In addition, soy isoflavones were not estrogenic enough to entrain gonadotropins in the postmenopausal women. Longer treatment times might reveal differences not apparent from this protocol. Our results suggest that in future research, identifying individuals with high sensitivities to isoflavones may play an important role in understanding the biological effects of phytoestrogens. GnRH challenge tests may be a useful tool to detect such individuals.

ACKNOWLEDGMENT

Thanks to Kathryn Sweeney, B.S.N. for her help during blood sampling procedures. Estraderm patches were supplied by Novartis Pharmaceuticals, East Hanover, NJ. GnRH (Factrel) was supplied by Wyeth-Ayerst (Philadelphia, PA)

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